Case report

De novo alpha 2 hemoglobin gene (HBA2) mutation in a child with hemoglobin M Iwate and symptomatic methemoglobinemia since birth

Marcos Borato Viana⁠a,*, André Rolim Belisário⁠b

⁠a Departamento de Pediatria da Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil
⁠b Centro de Tecidos Biológicos, Fundação Hemominas, Belo Horizonte, MG, Brazil

ABSTRACT

Cyanosis in an apparently healthy newborn baby may be caused by hemoglobin variants associated with the formation of methemoglobin, collectively known as M hemoglobins. They should not be confused with genetic alterations in methemoglobin reductase enzyme systems of red cells since treatment and prognosis are completely different. A newborn male child was noted to be significantly cyanotic at birth and is the basis for this report. Hemoglobin isoelectric focusing, acid and alkaline gel electrophoresis, and HBA/HBB gene sequencing were performed for the child, both parents and a sister. The newborn child was treated with methylene blue in an intensive care unit fearing that he had a defective reductase system and exposure to oxidant drugs or toxins. Newborn hemoglobin screening with high performance liquid chromatography was abnormal on the 10th and 45th days but no conclusive diagnosis was reached. Cyanosis persisted up to four years of age with no other symptoms. Hemoglobin M Iwate [alpha2 87(F8) His>Tyr, HBA2:c.262C>T] was detected. It was not present in the child’s presumed mother, father, sister, and brother. The analysis of 15 short tandem repeats in the trio demonstrated a de novo mutation occurrence (p-value < 1 × 10⁻⁶). The family was reassured that no further action was necessary and genetic counseling was provided. Methemoglobins should be considered for differential diagnosis of cyanosis in newborns even if no familial cases are detected. Except for cosmetic consequences, the clinical course of patients with hemoglobin M Iwate is unremarkable.

© 2014 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltd. All rights reserved.

*Corresponding author at: Av. Alfredo Balena, 190 - sala 267, Departamento de Pediatria da Faculdade de Medicina, Universidade Federal de Minas Gerais, 30130-100 Belo Horizonte, MG, Brazil.
E-mail address: vianamb@gmail.com (M.B. Viana).
1516-8484/$ - see front matter © 2014 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bjhh.2014.03.020
Introduction

Methemoglobinemia is the collective name for a clinical syndrome caused by an increase of methemoglobin in the blood. Broadly speaking, it may be caused by: i) a hemoglobin (Hb) variant that keeps the oxidized ferric state in the heme pocket and reduces oxygen affinity of the genetically altered Hb; ii) congenital deficiencies of nicotinamide adenine dinucleotide (NADH)-cytochrome b5 reductase, an essential enzyme needed to convert the heme ferric iron to its ferrous counterpart, thus making Hb apt to carry oxygen to the tissues; iii) an acute drug reaction to oxidizing agents that convert Hb to methemoglobin. By far the latter is the most common cause of the syndrome. Clinically all these three mechanisms will produce central cyanosis which is unresponsive to the administration of oxygen.¹

Amino acid substitutions within the heme pocket underlie all known methemoglobin (M-Hb) variants. Tyrosine replaces histidine in close proximity to the heme group at positions alpha 87 [F8] (Hb M Iwate), beta 92 [F8] (Hb Hyde Park), alpha 58 [E7] (Hb M Boston), or beta 63 [E7] (Hb M Saskatoon). Only in Hb M Milwaukee-I, the valine residue is replaced by glutamic acid at position beta 67 [E11] (http://globin.bx.psu.edu/hbvar/). For Hb M Iwate, as an example, substitution of the normal alpha His F8 side chain for a longer Tyr F8 stabilizes the deoxygenated T state and also reduces oxygen affinity of the native beta subunit.²

Children with alpha Hb M variants are symptomatic at birth because the interaction of the mutated alpha chain with the wild gamma chain is also abnormal and methemoglobin formation and cyanosis will follow. Beta variants are generally not detected at birth because Hb A concentration is low in newborns.

This report aims to describe in detail the clinical, hematological, and genetic features of a four-year-old boy with lifelong cyanosis who was proved to be heterozygous for a de novo mutation resulting in Hb M Iwate [alpha2 87(F8)His->Tyr HBA2:c.262C>T].

Methods

The boy’s family asked for the specialized opinion of one of the authors (MBV) about the cyanosis the child had shown since birth. Methemoglobinemia was diagnosed at that time but the etiology was not elucidated. The boy's father (33 years old) and mother (40 years old) were not consanguineous and had a normal daughter, three years older than the proband. Ethnic background included Italian, French, and Portuguese ancestry. Both parents signed an informed consent form in accordance with the Helsinki Declaration.

High performance liquid chromatography (HPLC) of Hb performed when the child was 10 and 45 days old were retrieved by the Blood Center of Ribeirão Preto, São Paulo, Brazil, and kindly sent to us to illustrate the present report. Hemoglobin electrophoresis was done in alkaline and acid media (SPIFE kits, Helena Laboratories, Beaumont, TX, USA) and by isoelectric focusing (IEF; Neonatal Hemoglobin Resolve Screen Kit, PerkinElmer Life and Analytical Sciences, Finland). Coulter T-890 was used to perform cell blood count.

Genomic DNA was isolated with the QiAamp® DNA Blood Mini Kit, QiAGEN. HBB, HBA2, and HBA1 genes were amplified with adequate specific primers. DNA sequencing was done in a ABI Prism 3130 Analyzer. A polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) test for the Hb M Iwate mutation was also performed. Endonuclease RsaI does not restrict the wild HBA2 gene while a restriction sequence (GT^AC) is created when the mutation is present.³ A multiplex gap PCR assay for alpha-thalassemia deletions was utilized.⁴

Parental testing was performed detecting 15 short tandem repeats (STR): D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA. A specific consent form to perform these tests was signed by both parents.

Results

The child was reported to be cyanotic immediately after birth. Apgar score was 8 and 10 in the first and fifth minute, respectively. Gestational age was 42 weeks and birth weight was 3.6 kg. Oxygen delivery was useless to revert the clinical state. Simple chest radiography, electrocardiogram, and echocardiogram were normal for the age. Although clinically well, the baby was transferred to an intensive care unit (ICU). Methemoglobinemia was diagnosed on the basis of a brown chocolate blood with a methemoglobin relative concentration of 12.5% (normal: below 5%). Methylene blue injections and oral vitamin C did not revert the cyanosis. He was discharged from the ICU after five days clinically healthy but with persistent cyanosis.

Activity of NADH cytochrome b5 reductase assay was not available anywhere in Brazil and so was not applied. HPLC chromatogram (beta thalassemia short program) when he was 45 days old is depicted in Figure 1. The relative concentrations of the peaks were: Hb F 28.4% at retention time of 1.16 min, Hb Aγ, 51.6% at retention time of 2.55 min, Hb A2 6.5% at retention time of 3.71 min, and unknown Hb 8.8% at retention time of 4.74 min. Although very faint to be graphically reproduced because of the time elapsed since the sample was drawn (ten days old) and the type of paper used for printing in the newborn screening, the HPLC (sickle cell short program) was similar to that when he was 45 days old. However, areas under the curve for the observed peaks were somewhat different portraying differences in gamma chain production between ten and 45 days of life. The unknown Hb concentration was 2.6% at retention time of 1.46 min and those for Hb F and Hb Aγ were 31.1% (retention time of 0.62 min) and 29.2% (retention time of 0.89 min), respectively. An unknown Hb between Hb F and Hb Aγ amounted to 12.3% and might represent the dimer alpha2/alpha2 (abnormal Hb F due to a mutated alpha chain).

Except for the mild to moderate cyanosis, the physical examination was completely normal when the proband was almost four years old. Finger pulse oximetry was 96%. His blood cell count was unremarkable: Hb concentration 13.3 g/dL, packed red cells 38.3%, mean corpuscular volume 84.2 fl, mean corpuscular hemoglobin 29.2 pg, reticulocyte count 1%, white cell count 9.1 x 10^9/L (57% neutrophils, 38% lymphocytes, 3% monocytes, and 2% eosinophils), platelets 334 x 10^9/L. Red
cell morphology was normal. Hematologic tests and pulse oximetry of other family members were also normal. Very recently a male brother was born with no cyanosis. Newborn screening tests were normal.

Figure 1 – High performance liquid chromatography chromatogram (beta thalassemia short program) when the child was 45 days old. The original line of the chromatogram was traced over to make it clearer 4 years after printing on a thermo-sensitive paper. The relative concentration of Hb A is already higher than that of Hb F, as expected. The estimated relative concentration of Hb M Iwate (area under the peak at the retention time of 4.74 min) is 8.8% [image kindly sent by the Blood Center of Ribeirão Preto, São Paulo, Brazil]

Figure 2 depicts alkaline and acid gel electrophoresis and IEF from the sample drawn for this report. Note that Hb M Iwate is clearly separated from Hb F and Hb A in both the alkaline gel procedure and IEF, but indistinguishable from Hb A in acid medium. The estimated relative concentration of Hb M Iwate read in the alkaline gel was 28%. Hb A2 concentration was 3% and Hb F 1.9%. In IEF, the color of Hb M Iwate is brown before trichloroacetic acid is applied to the IEF gel, in contrast to the vivid red color of Hb A (Figure 2C).

Figure 3A shows the electropherogram of the boy's HBA2 gene sequencing. PCR-RFLP with Rsal of all members of the family is depicted in Figure 3B. The mutation underlying heterozygous Hb M Iwate [alpha2 87(F8) His>Tyr HBA2:c.262C>T] was detected through sequencing and PCR-RFLP only in the proband. Homozygous thymidine (T/T) nucleotide polymorphism rs2541669 (NG_000006.1:g.33004 C>T) was detected in the proband and his father, and in heterozygous state (C/T) in his mother and sister (Figure 3B). The seven most common alpha-thalassemia deletion mutations were absent in all family members.

Parental testing yielded a combined paternity index of 221,784,816. The probability of paternity is, therefore, 99.999999%. The STR that contributed mostly to the index was D19S433 with a paternity index of 29.5899: the proband was genotyped as 14-16.2, his mother as 14-16, and his father as 14-16.2.

Discussion

Hereditary nigremia at Iwate Prefecture had been known by Japanese doctors for 160 years before a new M Hb was described in 1960 as the etiology of the disease. Since then there were several Japanese studies that have characterized the interesting chemical properties of Hb M Iwate. In the vivid description of the clinical picture by Professor Shibata, “The patients with this disease are cyanotic from childhood, looking like a man who has been swimming in a cold water pool for a long time”. As demonstrated in the present study, no ill effect on health has been documented before, even in patients who had lived to adulthood.

The amino acid substitution His>Tyr was demonstrated in 1964. The other names for Hb M Iwate as can be read at HbVar site (http://globin.bx.psu.edu/hbvar/) are M-Kankakei, M-Oldenburg, and M-Sendai (no reference found).

The mutation underlying Hb M Iwate in the HBA1 gene was reported only in 1987. Subsequently it was confirmed either in HBA1 or in HBA2. Specific amplification of the proband's alpha genes in the present study demonstrated that the mutation took place in HBA2. Homozygous T polymorphism rs2541669 (minor allele count T= 0.372/810) does not add any pathologic consequence as suggested by the normal clinical state of the homozygous T father.

On the basis of negative family histories for cyanotic manifestations there are three papers suggesting that, in
contrast to the many familial cases in Japan, a de novo mutation had occurred in patients from the USA, Italy, and Ireland. The present report is the first to clearly demonstrate that a de novo mutation was present in the proband. About one hundred de novo single-nucleotide germline mutations occur in an individual’s genome, the large majority without any deleterious effect for the embryo or child derived from the mutated germ cell. In this study genetic sequencing of HBA2 in the proband’s cells, other than blood, was not performed and so postzygotical somatic mosaicism has not been ruled out. Because new mutations can occur at any stage of gametogenesis, it is possible, although very unlikely, that our proband’s parents could procreate another child with Hb M Iwate. His mother was pregnant at the time of the diagnosis and, accordingly, realistic reassurance was provided to the couple. As mentioned before, a male baby was born and no cyanosis was detected.

Conclusion

M hemoglobins should be considered in the differential diagnosis of cyanosis in the newborn period even if no familial cases are detected. IEF strongly suggests M hemoglobins. Gene sequencing is necessary for the identification of abnormal variants. Except for cosmetic consequences, the clinical course of patients with Hb M Iwate is unremarkable.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors wish to thank the child’s family for agreeing to participate in the study. We also thank Maria Rosa de Almeida and Ivan de Lucena Angulo, from the Hemocentro de Ribeirão Preto, Brazil, for sending clinical and laboratory data when the child was recently born. This work was supported by Fapemig (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Brazil) and Fundação Hemominas. Marcos Viana has a research grant from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil. André Belisário has a scholarship from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and had a scholarship from FAPEMIG to be trained in sequencing.

REFERENCES